59th IWC Scientific Committee Meeting,
7-18 May 2007 Anchorage, USA.

A PRELIMINARY ASSESSMENT OF THE GENETIC DIVERSITY IN HUMPBACK WHALES (*Megaptera novaeangliae*) FROM ECUADOR AND POPULATION DIFFERENTIATION WITH OTHER SOUTHERN HEMISPHERE BREEDING GROUNDS AND FEEDING AREAS

FERNANDO FÉLIX[1,2], SUSANA CABALLERO[3,4] AND CARLOS OLAVARRÍA[3,5]

[1]Fundación Ecuatoriana para el Estudio de Mamíferos Marinos (FEMM). PO Box 09-01-11905, Guayaquil, Ecuador.
[2]Pontificia Universidad Católica del Ecuador. Av. 12 de Octubre y Patria, Quito Ecuador.
[3]Molecular Ecology and Evolution Laboratory. School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand.
[4]Present address: Pacific Biosystematics Research Laboratory, School of Science and Engineering Waikato University, Private Bag 3105, Hamilton, New Zealand.
[5]Fundación Centro de Estudios del Cuaternario Fuego, Patagonia y Antártica (CEQUA) c/o Instituto Antártico Chileno, Plaza Muñoz Gamero 1055, Punta Arenas, Chile.

E-mail: fernandofelix@femm.org

ABSTRACT

Information on the genetic characterization of humpback whales (*Megaptera novaeangliae*) breeding off Ecuador (2º10'S; 81º00'W; Stock G) is presented. Mitochondrial DNA was extracted and sequenced from forty three skin samples collected between 1994 and 2006 to establish the genetic diversity of Ecuadorian humpback whales. Samples were obtained either from beached animals (n=6), biopsies (n=1) or sloughed skin (n=38). Haplotype diversity ($h \pm SD$) was estimated to be 0.884 ± 0.042% and the nucleotide diversity ($\pi \pm SD$) 1.84 ± 0.96%. When compared with six Southern Hemisphere breeding grounds and two Stock G feeding areas, five new unique haplotypes were observed. One of the shared haplotypes was only recorded in the Western Australian breeding ground. The phylogenetic analysis showed that Ecuadorian haplotypes were distributed in three of the four described clades in the Southern Hemisphere AE, CD and IJ, being absent from the SH clade. A pair-wise Analysis of Molecular Variance (AMOVA) showed significant differentiation at both haplotype and nucleotide levels with all the Southern Pacific breeding grounds, except with Colombia. When compared with feeding areas, there were significant differences with Magellan Strait, but not with the Antarctic Peninsula. The AMOVA analysis suggests panmixia in the Stock G, but a larger sample is needed for a definitive conclusion.

KEY WORDS: humpback whale, genetics, breeding grounds, South America, Stock G

INTRODUCTION

It has been long known that humpback whales (*Megaptera novaeangliae*) breed during the austral winter along the northeastern coast of South America, off Colombia and Ecuador and further north off Panama (Kellog, 1929; Townsend, 1935; Mackintosh, 1942). Whales wintering off those neighbouring locations were considered to form a breeding single stock, despite no information supporting that assumption. Only in the last decade a first attempt was made to address this, when individually photo-identified whales were compared between those areas (Flórez-González *et al.*, 1998). Despite the reduced number of compared individuals from Ecuador, a few whales were matched, which suggested that interchange between these regions occurred. Recently, a small portion of humpback whales have been

identified wintering further north off Costa Rica (Acevedo-Gutiérrez and Smultea, 1995; Rasmussen *et al.*, 2007), which expand the range of the wintering grounds of this species in the Southeast Pacific. All those breeding areas have been linked to the feeding areas in the west side of the Antarctic Peninsula (Stone *et al.*, 1990; Garrigue *et al.*, 2002; Stevick *et al.*, 2004; Rasmussen *et al.*, 2007) and the Magellan Strait, in southern Chile (Acevedo *et al.*, 2007).

The discreteness of Southeast Pacific humpback whales was also assumed for a long time (Kellog, 1929; Mackintosh, 1942; Omura, 1957), despite again, the lack of evidence. Only recently, based on photo-identification (Garrigue *et al.*, 2002; Stevick *et al*., 2004) and genetic analyses (Caballero *et al*., 2001; Olavarría *et al*., 2007), the putative discreteness of this breeding stock (Stock G; IWC, 1998) was confirmed by comparisons with neighbouring Southern Hemisphere stocks.

Genetic studies have been conducted in recent years in different locations in the Southeast Pacific, including breeding grounds off mainland Ecuador and the Galapagos Islands (Félix *et al.*, 2006a), Gorgona Island and Málaga Bay in Colombia (Caballero *et al*., 2001; Olavarría *et al*., 2007) and feeding areas at the Magellan Strait in southern Chile (Olavarría *et al.*, 2006), and along the west coast of the Antarctic Peninsula (Olavarría *et al*., 2000). Such studies have provided an overview of the genetic diversity, which seems to be the lowest among humpback whale stocks in the Southern Hemisphere (Olavarría *et al*., 2007), and have shown a migratory relationship between the surveyed feeding areas in the Antarctic and the Colombian breeding grounds (Caballero *et al*., 2001; Olavarría *et al*., 2000; 2007). Interestingly, the whales inhabiting the Magellan Strait would represent a separate feeding aggregation, with their own genetic distinctiveness (Olavarría *et al*., 2006). The initial genetic analysis of samples collected off Ecuador showed that all the haplotypes were observed previously within the Southeast Pacific population, but further analysis was limited by the reduced number of collected samples (Félix *et al.,* 2006a).

In this report we expand our previous report (Félix *et al.,* 2006a) on the mitochondrial DNA (mtDNA) control region genetic diversity of humpback whales from Ecuador, including here new samples collected during the field season of 2006. Our results show that the genetic variability of this breeding ground is higher than previously reported. We also compared the genetic diversity from Ecuador with that from six Southern Hemisphere breeding grounds (Olavarría *et al*., 2007) and two Stock G feeding areas (Olavarría *et al*., 2006). We show a lack of differentiation between Ecuador and Colombia breeding grounds and the Antarctic Peninsula feeding area.

MATERIALS AND METHODS

Sampling

Humpback whale skin samples were obtained between 1994 and 2006 off Ecuador. Five samples were collected from beached animals, sixty from sloughed skin (Amos *et al.*, 1992) and one from biopsing with a Barnett crossbow equipped with a 60cm long arrow and modified tips (Lambertsen, 1987). Four beached animals from mainland Ecuador and the biopsied whale from Galapagos Islands were reported previously (Félix *et al*., 2006b). Sloughed skin samples were obtained between July and October 2006, onboard whalewatching vessels working at Salinas (2º10’S, 81º00’W; Figure 1). This sampling was conducted as part of a long-term research program on the coast of Ecuador by the Ecuadorian Foundation for the Study of Marine Mammals – FEMM (see Félix and Haase, 2001, 2005). An additional sample from a whale beached at Playas (2º38’S 80º23’W) in 2006 was also included in this analysis.

When sampling for sloughed skin, the whale-watching boat skippers were asked to approach the site where a whale hit the water and then small pieces of skin were picked up from the surface with a net attached to a pole. Pieces of skin were retrieved from the net with disposable toothpicks and saved into hermetic plastic containers with either a solution of DMSO saturated NaCl or ethanol 50%. The net was thoroughly washed with sea water until no pieces of skin were visible on its surface, and then the device was considered to be ready for the next sampling attempt. Sampled whales were photographed for individual identification either through flukes (see Katona *et al*., 1979) and/or dorsal fins, although positive identification did not occur in all cases. Once on shore, samples were stored at 4 °C for up to six months prior to laboratory analysis.

Molecular analysis

A fragment of approximate 800 bp of the mitochondrial DNA control region (CR) was amplified via the Polymerase Chain Reaction (Saiki *et al*., 1988) using standard reaction conditions (Palumbi, 1996). For the PCR, we used the primer combination t-Pro-whale Dlp1.5 (5'-TCACCCAAAGCTGRARTTCTA-3') and Dlp8 (5'CCATCGWGATGTCTTATTTAAGRGGAA-3') (Baker *et al*., 1998; Olavarría *et al*., 2007). To date, 43 samples have been successfully extracted, amplified and sequenced. The PCR profile was as follows: an initial denaturation at 95°C for 2 minutes, 36 cycles of 94°C for 30 seconds, 55°C for 1 minute and 72°C for 1 minute and 30 seconds, and a final extension at 72°C for 5 minutes. Free nucleotides and primers were removed from the PCR products using and Invitrogen PCR cleaning kit and directly sequenced in both directions using the standard protocols of Big Dye™ terminator sequencing chemistry on an ABI 3100 automated capillary sequencer (Perkin Elmer).

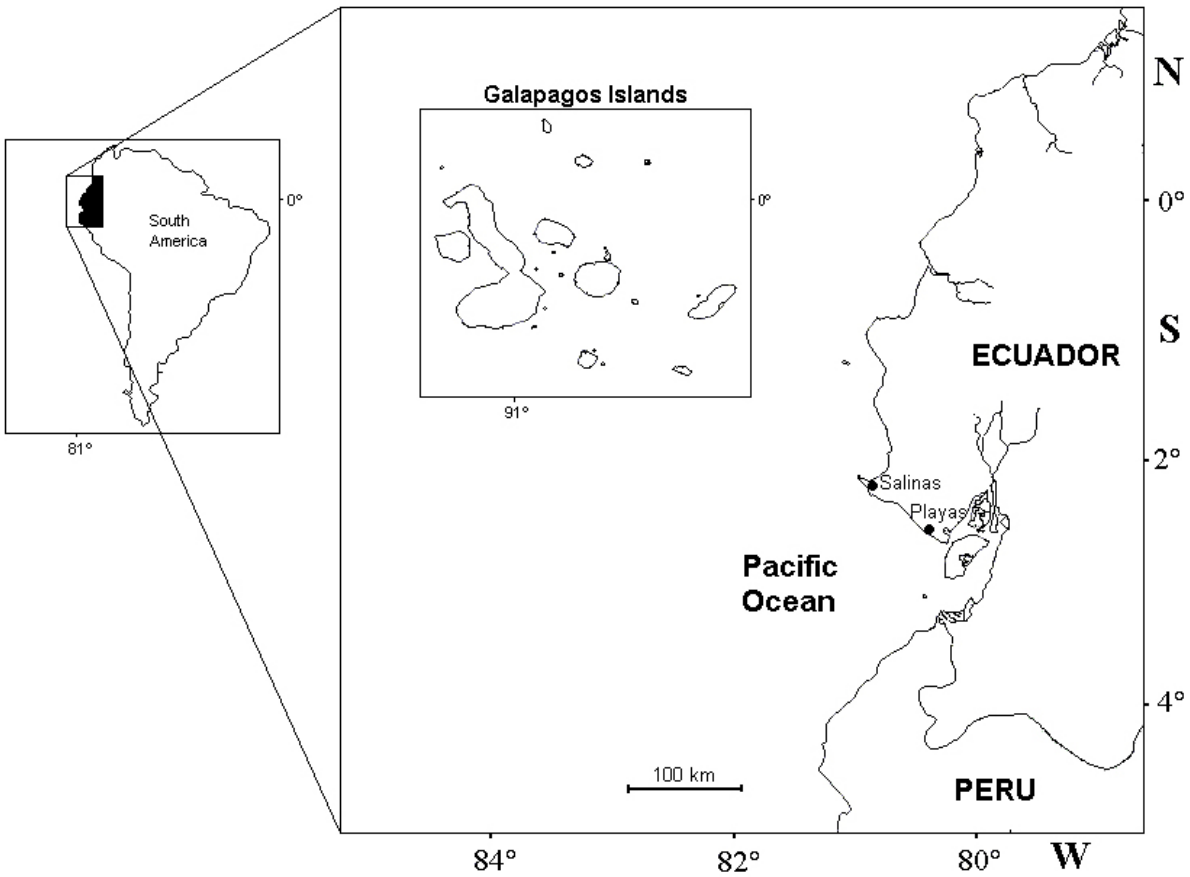

Figure 1. The surveyed areas in Ecuador and the Galapagos Islands.

All sequences were manually edited and aligned using Sequencher 4.1 software (Gene Codes Corporation). Sequences were trimmed to 469 bp to match a consensus region analysed previously (Olavarría *et al*., 2006; 2007). Control region haplotypes were defined using MacClade (Maddison and Maddison, 2000) and compared with haplotypes previously defined for other humpback whale populations in the South Pacific in order to define potential new haplotypes and haplotypes shared with other South Pacific breeding grounds and feeding areas. Haplotype nomenclature follows Olavarría *et al*. (2006, 2007). The software Arlequin Ver 2.000 (Schneider *et al*., 2000) was used to determine genetic diversity at both haplotype and nucleotide levels.

A phylogeny of humpback whale haplotypes was constructed using the Neighbor-Joining method implemented in PAUP* version 4.b10 (Swofford 2002, Sinauer Associates). For the Neighbor-Joining method, minimum evolution was used as the default optimality criterion. The sequences were adjusted for multiple substitutions using the Kimura 2-parameter model. Bootstrap support for Neighbor-Joining reconstruction was calculated after 100 simulations.

Geographic differentiation in haplotype frequencies and nucleotide variation between Ecuador and six other Southern Hemisphere breeding grounds and two Stock G feeding areas was quantified using an Analysis of Molecular Variance (AMOVA) (Excoffier, 1995) implemented in Arlequin software.

RESULTS

Genetic diversity and comparison with Southern Hemisphere breeding grounds and feeding areas

Twenty different haplotypes were determined in the 43 sequenced samples from Ecuador (Table 1). The variable nucleotides include two insertion/deletions, 29 transitions and 1 transversion. Haplotype diversity ($h \pm SD$) was estimated to be 0.884 ± 0.042% and the nucleotide diversity ($\pi \pm SD$) 1.84 ± 0.96%.

When compared with a total of 120 haplotype sequences from other Southern Hemisphere breeding grounds and feeding areas, 15 matched to previously reported haplotypes and five were new and unique for this stock. Only two of the previously found haplotypes (SP16 and SP42) had been not found in either a breeding ground or a feeding area of the Stock G. The proportion of unique haplotypes in the sample from Ecuador was significantly higher in respect to the proportion found in samples from Colombia and the Antarctic (X^2_2=8.16, p<0.05).

Ecuadorian haplotype frequencies were compared with other locations in the Southeast Pacific and the Antarctic Peninsula (Table 2). The most common haplotype reported in the Southeast Pacific and Antarctic Peninsula (SP90) was also the most common haplotype in Ecuador. The second more common haplotype found in Antarctic Peninsula and Colombia (SP32) was the third most common haplotype in Ecuador. However, the second more common haplotype in Ecuador (SP60) is barely represented in Antarctic Peninsula and in Colombia. Other common haplotypes in Antarctic Peninsula and Colombia (*e.g*. SP50 and SP98) were found in lower proportions in Ecuador, or were absent (SP52). Most of the haplotypes found in Ecuador were recorded once (n=12) or twice (n=5), which may be related to the small sample size.

One interesting finding was the presence of the haplotype SP16 in the Ecuadorian sample, which has been only reported in the Western Australian population (see Olavarría *et al*., 2007).

Table 1. Variable sites of 20 haplotypes found in 43 humpback whales mtDNA control region sequences from Ecuador (period 1994-2006). New unique haplotypes from Ecuador are named with the prefix MnEc. Insertions are indicated by a dash (-).

Haplotype	**Variable sites**
	111111111222222222333334
	268899222333466456666777881148 84
	423479168156738784567014577881 58
SP1	GTTCTA-TT-AACTCCTGTTCTTGTTCTTCCT
SP16	.C.TCCTCC-.G..TT....T..AC.....
SP25	.C.TCCTC.-.G..T.....T..A.....
SP26	.C.TCCTC.-.G..TT.....A.....
SP32	.C.T.C-C.-.G..TT....T..AC....T..
SP33	AC.T.C-C.-.G..TT....T..AC...C...
SP42	.CCT.C-..-GGT.TT....T..A....C...
SP50	.C...C-..-GGT.TT....T..A.C..C...
SP60	..CT.C-..-.GT.....CT....C.....C
SP61	...T.C-..C.GT..T...CT.....C
SP62	.....C-..-.G.....A.C..C.....T.
SP73	...T.C-..-.GTC..C.CC.C.....
SP90	A.....-..-.G.C.....T..
SP98	.....-..-.....T.....
Mno03Ma02	.....C-C.-GGT..T..C..C.A..TC.T..
MnEc52	.C.T.C-C.-.G..TT....T...C....T..
MnEc39	.....C-..-.G.....T.....
MnEc35	.....C-..-.G...TCA.C..C.....T.
MnEc32	.....-..-...C.....
MnEc25	.C.T.C-C.-.G..TT...CT...C....T..

Phylogeny of Southern Hemisphere humpback whales including the Ecuador breeding ground

The inclusion of Ecuador humpback whale haplotypes in a phylogeny of Southern hemisphere haplotypes showed that these were distributed in three out of the four major described clades (Olavarria *et al.*, 2007). Most haplotypes belonged to the CD (67.4%) clade, followed by IJ (30.3%) and AE (2.3%) clades, being absent from the SH clade (Figure 2).

Population differentiation analysis compared with other South Pacific breeding grounds and feeding areas

A pair-wise AMOVA of Ecuadorian humpback whale haplotypes showed significant differentiations at both haplotype and nucleotide levels with all the breeding grounds, except with Colombia. When compared with feeding areas, there were significant differences with Magellan Strait, but not with the Antarctic Peninsula, at both levels (Table 3).

Table 2. Comparison of haplotype diversity and occurrence between samples from Ecuador and other two sites surveyed in the Southeast Pacific and Antarctic (Data for other sites from Olavarría *et al*. 2006 and 2007).

Haplotype (469 pb)	**Ecuador *n***	**Colombia *n***	**Antarctic *n***	**Magellan St *n***
SP1	2	5	7	
SP8		2	2	4
SP9		1		
SP10		4	4	
SP12		1	1	
SP14		4	1	
SP16	1			
SP25	2	1		
SP26	1			
SP29		1		
SP32	3	17	8	
SP33	1	6		
SP42	2			
SP43		2		
SP50	1	11	6	
SP52		9	7	
SP54		1		
SP60	4	5	2	
SP61	2	1	2	
SP62	2	7	2	
SP63		4	2	
SP66		3	1	
SP68		1	3	
SP72		2	1	
SP73	1	2	2	
SP74		1		
SP75		1		
SP88			2	
SP90	14	38	21	42
SP98	1	10	6	1
SP100			4	
PS101		8		
SP111			1	
Mno03MA02	1			5
MnoEc25	1			
MnoEc32	1			
MnoEc35	1			
MnoEc39	1			
MnoEc52	1			
Mno96AP07			1	
Mno97AP06			1	
Mno97AP07			1	
Mno99AP09			1	
Total	***43***	***148***	***89***	***52***

Table 3. Pair-wise test of differentiation for mtDNA control region sequence between Ecuador and Colombia (Col), French Polynesia (FP), Cook Islands (CI), Tonga (Tg), New Caledonia (NC) and Western Australia (WA) breeding grounds, and the Antarctic Peninsula (AP) and Magellan Strait (MSt) feeding areas based on F_{ST} and Φ_{ST} indexes. Unadjusted p-values based on 5,000 random permutations are shown in italics. Values in bold are significantly greater than those found in 5% of the 5,000 random permutations of the data matrix ($p<0.05$). The division of breeding stocks (D - G) follows the model of stock structure currently in discussion by the Scientific Committee of the International Whaling Commission (IWC, 2005).

		Col	FP	CI	Tg	NC	WA	AP	MSt
	Stock	*G*	*F2*	*F1*	*E3*	*E2*	*D*	*G*	*G*
Ecuador	F_{ST}	**0.0046**	0.0881	0.0839	0.0662	0.0662	0.0635	**0.0042**	0.1753
	p-value	***0.1797***	*< 0.0001*	*< 0.0001*	*< 0.0001*	*< 0.0001*	*< 0.0001*	***0.1797***	*< 0.0001*
	Φ_{ST}	**-0.0070**	0.0543	0.0560	0.0431	0.0403	0.0408	**-0.0053**	0.1834
	p-value	***0.8046***	*0.0012*	*0.0008*	*0.0008*	*0.0014*	*0.0014*	***0.6852***	*< 0.0001*

DISCUSSION

Research on the Ecuadorian humpback whale breeding ground has been undertaken in several locations along the coast of Ecuador since 1991, including, among others, behavioural studies, distribution and population assessments (Scheidat *et al*., 2000; Félix and Haase, 2001, 2005; Castro and González, 2002; Félix *et al*., 2006b). Analyses presented here are the first attempt to assess the genetic differentiation among breeding grounds of the Stock G, in order to obtain insights into the potential panmixis in this stock.

Genetic diversity

Our current analyses show that most sampled individuals in mainland Ecuador and Galapagos share haplotypes with whales from Colombia, Magellan Strait and the Antarctic Peninsula. As it was expected, the most common haplotype found in the samples from Ecuador (SP90) is the most common haplotype found in those sampled sites. This haplotype has not been recorded in other stocks of the Southern Hemisphere (Olavarría *et al*., 2007), which suggest that this is a characteristic of the Stock G along all breeding grounds and feeding areas.

The presence of haplotype SP16 in the sample from Ecuador, which has been found only in the Western Australian population, is puzzling. According to Olavarría *et al*. (2007), the phylogenetic relationship among the humpback whale stocks in the South Pacific diverge with a west-east direction. This explains why the Southeast Pacific stock, geographically more distant from its westernmost neighbour (Stock F at French Polynesia) than the remaining southern stocks, has the greatest genetic differentiation of all southern Pacific stocks (Olavarria *et al*., 2007). Although the absence of haplotype SP16 in the samples from the Eastern and Central Pacific could be due to sampling bias, an alternative explanation would be a genetic flow from far distant Western Australian whales. It has been demonstrated that extensive movement across stocks occur, as shown by the movement of marked whales from Western Australia into de South Pacific (Chittleborough, 1965) and of individually identified whales across the South Pacific (SPWRC, 2006) and between the Indian and Atlantic basin (Pomilla and Rosembaum, 2005). A similar flow could occur with humpback whales from the Southeast Pacific, however no matches have been found using photo-ID neither with whales from the western and central Pacific (Garrigue *et al.,* 2002) nor the Western Atlantic (Stevick *et al*., 2004). Collaboration between research groups working with humpback whales in the Southern Hemisphere, such as Rosenbaum *et al*. (2006), is necessary in order to understand the genetic flux of the species at hemispheric scale.

Panmixia within the Stock G breeding grounds, and genetic relationship with the feeding areas and other Southern Hemisphere breeding grounds

The AMOVA analysis suggest that panmixia may be the case in the Stock G, when comparing the breeding areas in Colombia (Gorgona Island and Málaga Bay, located just 200nm north from our study area) and Ecuador. This lack of genetic differentiation between Colombia and Ecuador breeding grounds has been observed partially at demographic level (Flórez-González *et al.*, 1998), however, a more thorough comparison is needed, including the full catalogues from both areas.

Interestingly, Ecuador and Colombia breeding grounds shared their genetic relationship with the Antarctic Peninsula, suggesting this as the main feeding area for this stock. Similarly, both breeding grounds differentiated from the Magellan Strait, supporting that heterogeneity occurs in the migratory pattern of the Stock G at the feeding areas (Olavarría *et al.*, 2006).

The significant differences at haplotype and nucleotide levels with other Southern stocks confirm the discreteness of the Southeast Pacific humpback whale stock as previously reported (Olavarría *et al*., 2007) and assumed for a long time (Kellog, 1929; Mackintosh, 1942; Omura, 1957).

Further considerations and analyses

This was the first time that sloughed skin was collected aboard whalewatching vessels during our long-term study. Although it implicated approaching the animals at shorter distance than allowed for whalewatching boats, it was relative easy and convenient. To our knowledge, this form of collecting sloughed skin has not been tried elsewhere, as whalewatching regulations prevent in most cases the approach to less than 100m from humpback whales (Carlson, 2004). We recognize, however, that the convenience of sampling whales in this form should be carefully evaluated under a scientific perspective to prevent it becomes a pretext to get a closer look of the whales by commercial boats. As sampling based on sloughed skin is biased towards individuals with surface activity, and maybe affecting age/sex class representativeness in the sample, it is unknown how such bias may affect the interpretation of genetic analysis. Also, some whales may be less shy to approaching boats, causing additional bias (for example adult males vs. females with calves). All these sources of heterogeneity need further consideration. Anyway, sloughed skin is considered safe, a source of good material for mitochondrial DNA analyses and an alternative to biopsying (Clapham *et al*., 1993).

Previous studies have shown that a low within-year resighting rate and low birthing rate is found in coastal waters of Ecuador (Scheidat *et al*., 2000; Félix and Haase 2001; Félix *et al*., 2007), suggesting that most whales recorded in this southern part of the breeding area would be in transit. It is necessary to extend the sampling into other northern locations in mainland Ecuador, as well as at the offshore Galapagos Islands. This would help to obtain a more representative sample from this location.

Further analyses should take into consideration the potential effect of re-sampling within Ecuador. Not all sampled whales were individually identified; however, based on previous matching rates of photo identified whales between Ecuador and Colombia (Florez-González *et al*., 1998) and between Ecuador and Antarctic Peninsula (Stevick *et al*., 2004), resampling would be as high as 10% on the average (8.3% with Colombia and 12.6% with Antarctic). Within year duplicity of samples taken in 2006 would be even lower, as the intra-yearly resighting rate was 2% (Félix *et al*., 2007).

ACKNOWLEDGEMENTS

The authors would like to thank skippers and tourist operators in Salinas for their patience during the sampling. Jéssica Falconí and Andrea Auger assisted in the field. The humpback whale research in Ecuador is supported by the Whale and Dolphin Conservation Society (WDCS).

REFERENCES

Acevedo-Gutiérrez, A. and M.A. Smultea. 1995. First records of humpback whales including calves at Golfo Dulce and Isla del Coco, Costa Rica, suggesting geographical overlap of northern and southern hemisphere populations. *Marine Mammal Science* 11:554-560.

Acevedo, J., Rasmussen, K., Félix, F., Castro, C., Llano, M., Secchi, E., Saborío, M.T. Aguayo-Lobo, A., Haase, B., Scheidat, M., Dalla-Rosa, L., Olavaria, C., Forestell, P., Acuña, P., Kaufman, G., and Pastene, L. (2007). Migratory destinations of the humpback whales from Magellan Strait feeding ground, Chile. *Marine Mammal Science* 23(2): 453-463.

Amos, W., H. Whitehead, M.J. Ferrari, D.A. Glockner-Ferrari, R. Payne and J. Gordon. 1992. Restrictable DNA from sloughed cetacean skin: its potential for use in population analysis. *Marine Mammal Science* 8:275-283.

Baker, C. S., Flórez-González, L., Abernethy, B., Rosenbaum, H. C., Slade, R.W., Capella, J., and Bannister, J. L. 1998. Mitochondrial DNA variation and maternal gene flow among humpback whales of the Southern Hemisphere. *Marine Mammal Science* 14(4):721-737.

Caballero, S., Hamilton, H., Jaramillo, H., Capella, J., Flórez-González, L., Olavaria, C., Rosembaum, H. C., Guhl, F. and Baker, C. S. 2001. Genetic characterization of the Colombian Pacific coast humpback whale population using RAPD and mitochondrial DNA sequences. *Memoirs of the Queensland Museum* 47(2):459-464.

Carlson, C. 2004. A review of whale match guidelines and regulations around the World, version 2004. IFAW. 133p. Available from: http://www.iwcoffice.org/_documents/sci_com/WWregsApril03.pdf.

Castro, C. and J. González. 2002. Población de la ballena jorobada *Megaptera novaeangliae*, Balaenopteridae, en el Parque Nacional Machalilla, Ecuador. Page 230. Universidad Central del Ecuador, Quito.

Chittleborough, R.G. 1965. Dynamics of two populations of humpback whales, Megaptera novaeangliae (Borowski). *Australian Journal of Marine and Freshwater Research* 16:33-128.

Clapham, P. J., Palsboll, P. J. and Matilla, D. K. 1993. High-energy behaviors in humpback whales as a source of sloughed skin for molecular analysis. *Marine Mammal Science*, 9:213-220.

Excoffier, L. 1995. AMOVA: Analysis of Molecular Variance. University of Geneva, Geneva

Félix, F. and Haase, B. 2001. The humpback whale off the coast of Ecuador, population parameters and behavior. *Revista de Biología Marina y Oceanografía*, 36(1):61-74.

Félix, F. and Haase, B. 2005. Distribution of humpback whales along the coast of Ecuador and management implications. *Journal of Cetacean Research and Management.* Vol. 7(1):21-31.

Félix, F., Palacios, D., Caballero, S., Haase, B. and Falconí, J. 2006a. The 2005 Galápagos humpback whale expedition: a first attempt to assess and characterize the population in the archipelago. Document SC/A06/HW15 presented in the International Whaling Commission Workshop on Comprehensive Assessment of Southern Hemisphere Humpback Whales, Hobart, Tasmania: 3-7 April 2006. 7p.

Félix, F., Castro, C., Haase, B., Forestell, P., Álava, J. J., and Scheidat, M. 2006b. Estimates of the Southeastern Pacific humpback whale stock with mark-recapture models in Ecuador. Document SC/A06/HW13 presented in the International Whaling Commission Workshop on Comprehensive Assessment of Southern Hemisphere Humpback Whales, Hobart, Tasmania: 3-7 April 2006. 7p.

Félix, F., J. Falconí, A. Auger, B. Haase & P. Jiménez. 2007. Scientific and educational activities in Ecuador during 2006. Final Report to Whale and Dolphin Society WDCS, England. Guayaquil, Ecuador, January 2007. 24 p. (Unpublished).

Flórez-González, L., J. Capella A., B. Haase, G. A. Bravo, F. Félix, & T. Gerrodette. 1998. Changes in winter destinations and the northernmost record of southeastern Pacific humpback whales. *Marine Mammal Science* 14(1):189-196.

Garrigue, C., A. Aguayo, V.L.U. Amante-Helweg, C.S. Baker, S. Caballero, P. Clapham, R. Constantine, J. Denkinger, M. Donoghue, L. Flórez-González, J. Greaves, N. Hauser, C. Olavarría, C. Pairoa, H. Peckham and M. Poole. 2002. Movements of humpback whales in Oceania, South Pacific. *Journal of Cetacean Research and Management* 4:255-260.

IWC. 1998. Report of the Scientific Committee. *Reports of the International Whaling Commission*. 48:53-118.

IWC. 2005. Report of the Scientific Committee, Annex H, Report of the Sub-Committee on other Southern Hemisphere whale stocks. *Journal of Cetacean Research and Management* 7 (Suppl.):235-246

Katona, S., Baxter, B., Brazier, O., Kraus, S., Perkins, J. and Whitehead, S. 1979. Identification of humpback whales by fluke photographs. Pages 33-44 in H.E. Winn and B.L. Olla, eds. Behavior of Marine Animals. Plenum Press, New York.

Kellogg, R. 1929. What is known of the migration of some of the whalebone whales. *Smithsonian Institution Annual Report* 1928:467-494.

Lambertsen, R.H. 1987. A biopsy system for large whales and its use for cytogenetics. *Journal of Mammalogy* 68:443-445.

Mackintosh, N.A. 1942. The southern stocks of whalebone whales. *Discovery Reports* 22:197-300.

Maddison, D. R. and Maddison, W. P. (2000) *MacClade: analysis of phylogeny and character evolution*. Sinauer, Sunderland, MA, USA.

Olavarría C., Baker C. S., Medrano, L., Aguayo-Lobo, A., Caballero, S., Flórez-González, L., Capella, J., Rosenbaum, H. C., Garrigue, C. and Graves, J. 2000. Stock identity of Antarctic Peninsula humpback whales inferred from mtDNN variation. Document SC/52/IA15 submitted to the Scientific Committee of the International Whaling Commission. Adelaide, Australia, June 2000.

Olavarría, C., Aguayo, A., Acevedo, J., Medrano, L., Thiele, D. and Baker, C. S. 2006. Genetic differentiation between two feeding areas of the Eastern South Pacific humpback whale population: Update on SC/57/SH3. Document SC/A06/HW29 presented in the International Whaling Commission Workshop on Comprehensive Assessment of Southern Hemisphere Humpback Whales, Hobart, Tasmania: 3-7 April 2006. 7p.

Olavarría, C., Baker, C.S., Garrigue, C., Poole, M., Hauser, N., Caballero, S., Flórez-González, L., Brasseur, M., Bannister, J., Capella, J., Clapham, P., Dodemont, R., Donoghue, M., Jenner, C., Jenner, M. N., Moro, D., Oremus, M., Paton, D., Rosenbaum, H. and Russell, K. 2007. Population structure of South Pacific humpback and the origin of the eastern Polynesian breeding grounds. *Marine Ecology Progress Series* 330:257-268.

Omura, H. 1953. Biological study on humpback whales in the Antarctic whaling areas IV and V. *Scientific Report of the Whales Research Institute* 8:81-102.

Palumbi, S. R. 1996. Nucleic acids II: the polymerase chain reaction. In: Molecular Systematics (eds, Hillis D, Moritz C, Mable BK) Sinauer Associates, Suderland, MA.

Pomilla, C. and Rosenbaum, H. 2005. Against the current: an inter-oceanic whale migration event. *Biology Letters* 1:476-479.

Rasmussen, K., D.M. Palacios, J. Calambokidis, M.T. Saborio, L.D. Rosa, E.R. Secchi, G.H. Steiger, J.M. Allen and G.S. Stone. 2007. Southern Hemisphere humpback whales wintering off Central America: insights from water temperature into the longest mammalian migration. *Biology Letters* doi:10.1098/rsbl.2007.0067.

Rosenbaum, H.C., C. Pomilla, C. Olavarria, C.S. Baker, M.C. Leslie, M.C. Mendez, S. Caballero, M. Brassuer, J. Bannister, P.B. Best, S. Bonatto, T. Collins, M.H. Engel, P.J. Ersts, K.P. Findlay, L. Florez-Gonzalez, C. Garrigue, N. Hauser, C. Jenner, M. Meyer, G. Minton, M. Poole and Y. Razafindrakoto. 2006. A first and preliminary analysis of mtDNA sequences from humpback whales for breeding stocks A-G and X. Report SC/A06/HW59 to the Inter-sessional workshop for the Comprehensive Assessment of Southern Hemisphere humpback whales Scientific Committee of the International Whaling Commission. Hobart, 3 - 7 April 2006.

Saiki, R.K., Gelfand, D.H., Stofell, S., Scharf, R., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science,* 239**,** 487-491.

Scheidat, M., Castro, C., Denkinger, J., González, J. and Adelung, D. 2000. A breeding area for humpback whales (*Megaptera novaeangliae*) off Ecuador. *Journal of Cetacean Research and Management* 2(3):165-171.

Schneider, S., Roessli, D. and Excoffier, L. 2000 Arlequin: A software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

SPWRC, C. Garrigue, C.S. Baker, R. Constantine, M. Poole, N. Hauser, P. Clapham, M. Donoghue, K. Russell, D. Paton and D. Mattila. 2006. Interchange of humpback whales in Oceania (South Pacific), 1999 to 2004. Report SC/A06/HW55 to the Inter-sessional workshop for the Comprehensive Assessment of Southern Hemisphere humpback whales Scientific Committee of the International Whaling Commission. Hobart, 3 - 7 April 2006.

Stevick, P., Aguayo, A., Allen, J., Avila, I. C., Capella, J., Castro, C., Chater, K., Engel, M. H., Félix, F., Flórez-González, L., Freitas, A., Haase, B., Llano, M., Lodi, L., Muñoz, E., Olavarría, C., Secchi, E., Scheidat, M. and Siciliano, S. 2004. A note on the migrations of individually identified humpback whales between the Antarctic Peninsula and South America. *Journal of Cetacean Research and Management.* 6(2):109-113.

Stone, G., Flórez-González, L. and Katona, S. 1990. Whale migration record. *Nature* 346: 705.

Swofford, D. L. 2002. PAUP: phylogenetic analysis using parsimony, 4.0b10. Florida State University, FL.

Townsend C. H. 1935. The distribution of certain whales as shown by logbook records of American whaleships. *Zoologica NY* 16:133-144.

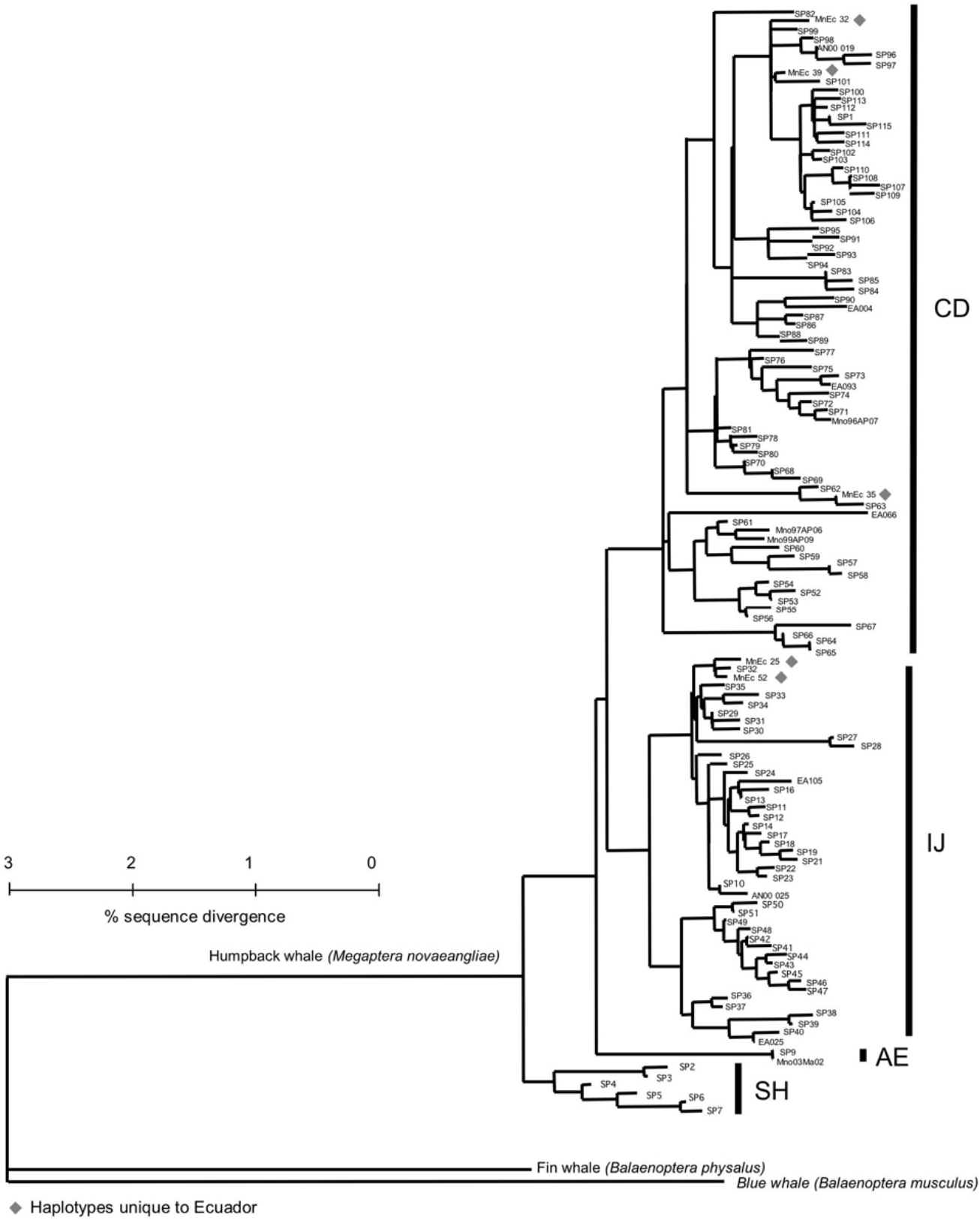

Figure 2. Phylogenetic reconstruction of 125 humpback whales mtDNA control region haplotypes (469 bp in length) using Neighbor-Joining and Kimura 2-parameter distances. Bootstrap support (after 100 simulations) for major clades (AE, CD, IJ, SH) indicated above branch when > 50%; fin and blue whale sequences included as outgroups.